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## The alloantigen-independent factors brain death and cold ischemia

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## **Chapter 5:**

# **Donor Dopamine Treatment in Brain Dead Rats is Associated with an Improvement in Renal Function early after Transplantation and a Reduction in Renal Inflammation**

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## **ABSTRACT**

**Background** Brain death is associated with tissue inflammation. Since dopamine treatment of brain dead donor rats reduces renal monocyte infiltration, we tested if this treatment affects renal function and inflammation in recipients.

**Methods** Brain death was induced in F344 rats and was maintained for 6 hrs in all experiments. Dopamine was given for 6 (DA 6) or 3 hours (DA 3) from the onset of brain death on. Ventilated non brain dead (NBD) and brain dead (BD) animals served as controls. Kidneys were transplanted into bilaterally nephrectomized LEW recipients. Serum creatinine (s-crea) was measured and leukocyte infiltration was assessed 10 days after transplantation.

**Results** One day after transplantation s-crea was significantly reduced in recipients that received a renal allograft from dopamine treated BD or from NBD rats compared to BD vehicle ( $P<0.05$ ). 10 days after transplantation, the number of infiltrating monocytes was significantly lower in grafts obtained from dopamine treated and from NBD rats ( $P<0.05$ ). A reduced infiltration in these grafts was confirmed by Banff 97 classification. CINC1 and IL-6 mRNA expression were reduced in DA rats compared to BD controls. No difference for MCP-1 and IL-10 were found.

**Conclusions** These findings may explain the salutary effect of donor dopamine treatment in renal transplantation.

## INTRODUCTION

Although brain death is considered to be an important cause of pre-transplantation allograft injury, the majority of renal allografts are still retrieved from deceased donors. Therefore, understanding the mechanisms causing tissue injury in BD donors and investigating possible strategies to overcome or prevent these harmful processes in BD are essential. Since brain death promotes inflammation in end-organs, affects hormone regulation and hemodynamic stability, it is generally accepted that this conditions severely influences organ quality [1-3]. Brain death seems to be associated with a worse ischemia/reperfusion injury after transplantation [4], although in large animals this could not be demonstrated [5, 6]. In the sequel of brain death a rapid up-regulation of inflammatory mediators like IL-6 and TNF $\alpha$  occurs [7-10]. This might in turn result in the upregulation of an array of genes including selectins, fibrinogen and KIM-1 [11]. Brain death is considered to be a risk factor for organ dysfunction [12, 13] and may accelerate acute rejection episodes [14-16].

Because damaging process in organ allografts already occurs during brain death, donor treatment might represent a genuine approach to improve organ quality. Several experimental studies have emphasized the applicability of this approach and unambiguously demonstrated its benefit on transplantation outcome. The use of agents that induce endogenous HO-1 expression [17] or the use of anti-inflammatory agents [18], i.e. P-selectin glycoprotein ligand (sPSGL) or steroids, seems to be promising in this regard.

In two retrospective clinical studies, Schnuelle et al. [19, 20] demonstrated that dopamine treatment of brain dead donors have a beneficial effect on delayed-graft function and long-term renal allograft survival. The favourable effect of donor dopamine treatment might be related to its anti-inflammatory properties, since dopamine inhibits the production of chemokines in renal tubular epithelial and endothelial cells [21]. In addition, dopamine treatment of brain dead rats reduces monocyte infiltration and also significantly improves mean arterial pressure and organ perfusion [22, 23]. Since clinical studies have shown that donor dopamine treatment positively affects delayed graft function and acute rejection, the present study was conducted to address if dopamine treatment of brain dead donor rats can influence early renal function and renal inflammation after transplantation. To this end, we harvested renal allograft from brain dead Fisher rats and transplanted these in Lewis recipients. Renal function and histology were assessed in both the donor and the recipient.

## METHODS

### *Animals*

Inbred male Lewis (LEW, RT1<sup>l</sup>) and Fisher (F344, RT1<sup>lv</sup>) rats weighing 200 to 250 g were obtained from Charles River (Sulzfeld, Germany). Animals were kept under standard conditions and fed standard rodent chow and water ad libitum. All procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences and were approved by the local authorities (RP Karlsruhe, AZ 35–9185.81/27/04).

### *Experimental Protocol*

Before induction of brain death, donor animals were anesthetized with ketamine (Ketanest, Pfizer, Karlsruhe, Germany, 100 mg/kg intraperitoneally) and xylazine (Rompun, BayerVital, Leverkusen, Germany, 6 mg/kg intraperitoneally) and placed on a heating table to keep their body temperature constant. A 3F Fogarty catheter was inserted epidural in an occipital burr hole and gradually inflated during 1 minute with 200 µL of saline. The state of brain death was verified by the occurrence of autonomic storm, the absence of corneal reflexes and by an apnoea test. All animals were mechanically ventilated by a tracheostoma with a rodent ventilator (Ugo Basile, Comerio, Italy). Systemic blood pressure (mean arterial pressure [MAP], mm HG) was continuously measured (6 hrs) in the donors by a femoral arterial catheter (Statham pressure transducer P23Db and a Gould pressure processor, FMI, Ober-Beerbach, Germany). Anesthetized, non brain-dead ventilated donor animals served as controls. Recipients were anesthetized with enflurane (Ethrane; Aca Mueller/Adag Pharma, Gottmadingen, Germany). Experiments were performed in the allogeneic Fisher-Lewis rat model. Animals were divided into 5 groups. Donor animals were treated intravenously by microinjection pumps (CMA/100, CMA/ Microdialysis, Sweden) according to the following scheme:

**Group 1:** Fisher donor rats were ventilated and treated intravenously with NaCl 0.9% for 6 hrs (non brain death (NBD) group).

**Group 2:** Brain death was induced in Fisher donor rats. BD lasted for 6 hrs; the animals were ventilated and treated with NaCl 0.9% (brain death (BD) group).

**Group 3:** Brain death was induced in Fisher donor rats. BD lasted for 6 hrs; the animals were ventilated and treated with NaCl 0.9% and HES (hydroxy ethyl starch) to normalize blood pressure (brain death normotensive (BD-normot) group).

**Group 4:** Brain death was induced in Fisher donor rats. BD lasted for 6 hrs; the animals were

ventilated and treated for 3 hrs with 10 µg/min/kg dopamine [23] (dopamine treated group (DA 3)).

**Group 5:** Brain death was induced in Fisher donor rats. BD lasted for 6 hrs; the animals were ventilated and treated for 6 hrs with 10 µg/min/kg dopamine (dopamine treated group (DA 6)).

Infusion of dopamine and control solutions started at the beginning of brain death induction. In each group, the kidney was harvested after 6 hours, flushed with 1 ml of cold UW solution and transplanted in allogeneic bilateral nephrectomized Lewis rats. The transplantation was performed as previously published [24-26]. No immunosuppression was administered. Each group consisted of a minimum of six animals.

### ***Renal function***

Renal function was assessed both in donors and recipients by serum creatinine. In the recipients serum creatinine was measured on days 0, 1, 3, 5, 8 and 10 after transplantation, while in the donors serum creatinine was measured before induction of brain death and at the end of the brain death period.

### ***Immunohistochemistry***

Renal grafts were harvested 10 days after transplantation. The upper pole of the kidney was frozen in liquid nitrogen, and the remaining part fixed in 10% buffered formalin solution. Serial sections (4 µm) of paraffin embedded tissue were fixed in 10% neutral buffered formalin for immunohistochemical staining. The sections were extensively washed with phosphate-buffered saline (PBS) and subsequently treated with 3% hydrogen peroxide. Endogenous biotin activity was blocked using the Avidin blocking kit, (Vector, Burlingame, CA). Monocytes and macrophages were detected by ED1 (monoclonal mouse anti-rat, (Linaris Biologische Produkte GmbH, Germany) and by major histocompatibility complex (MHC) class II expression (F-17-23-2, monoclonal mouse anti-rat, Linaris). Incubations of Primary and secondary antibodies were sequentially applied and to the sections for 1 hr. After each incubation step the sections were extensively washed with PBS. Standard avidin-biotin complex staining was performed according to the manufacturer's instructions (ABC kit, Vector). After addition of 3,3' diaminobenzidine substrate and washing the sections were counterstained with hematoxylin. Evaluation of ED1 and MHC class II positive cells was performed in a blinded fashion at 400× magnification. At least six animals per group and 20 fields per section were analysed.

### ***Light microscopy***

Paraffin sections were stained with hematoxylin-eosin, periodic acid-Schiff, and trichrome. A minimum of 20 microscopic fields per graft were assessed. Histologic grading was performed according to the Banff '97 classification [27]. Sections were blindly evaluated and graded by a renal pathologist (R.W.). Histologic evaluation and grading included transplant glomerulopathy, tubulointerstitial fibrosis, tubular atrophy, and vasculopathy. The histologic grading scale was from 0 to 3 (0=not present, 1=mild alteration, 2=moderate alteration, and 3=severe alteration).

### ***Histomorphometric analysis***

Hematoxylin-eosin stained sections were used to determine glomerular size.

The glomerular volume ( $V_g$ ) was calculated from the mean planar area of glomeruli of which the glomerular tuft and the macula densa could be seen. At least ten glomeruli per section were evaluated. Mean glomerular area ( $A_g$ ) was estimated by the surface calculating tool of analySIS. Volume was calculated according to the Weibel and Gomez method [28, 29]:

$$V_g = A_g^{3/2} * \beta / d$$

in which the shape coefficient of the sphere ( $\beta$ ) is 1.38 and the size distribution of the glomeruli ( $d$ ) is 1,01\_representing the size assuming a 10% coefficient of variation of the caliper diameter.

***Light cycler polymerase chain reaction***

Grafts from ventilated non BD-, NaCl treated and dopamine treated BD rats were investigated 10 days after transplantation. Snap-frozen tissue samples were homogenized using a Polytron homogenizer (IKA Labortechnik/Fischer Scientific). 500 ng of total RNA was reversed transcribed into cDNA according to the manufacturer's instructions, using the 1<sup>st</sup> Strand cDNA Synthesis Kit. cDNA was diluted in 20 µl DEPC-treated water and stored at -80°C until use. Specific DNA standards were generated by PCR amplification of cDNA, purification of the amplified products, and quantification by spectrophotometry. Light cycler PCR of cDNA specimen and DNA standards were conducted in a total volume of 25 µl, containing 2 µl FastStart DNA Master SYBR GreenI, 10 pMol of each forward and reverse primer and 2mMgCl<sub>2</sub>. Primer sequences were as follows: Cytokine Induced Neutrophil Chemoattractant 1 (CINC-1) (forward: "AGT TTG AAG GTG ATG CCG C 3'", reverse: 5`GGA CAC CCT TTA GCA TCT TTT G 3`), Interleucin 6 (IL6) (forward: 5` GAT ACC ACC CAC AAC AGA CCA G 3', reverse: 5` GCC ATT GCA CAA CTC TTT TCT C 3`), Macrophage Chemoattractant Protein 1 (MCP-1) (forward: 5`CAG ATG CAG TTA ATG CCC CA 3`, reverse: 5`CCT GCT GCT GGT GAT TCT CTT 3`) and Interleucin 10 (IL10) (forward: 5`TAC CTG GTA GAA GTG ATG CCC C 3`, reverse: 5`AAT CGA TGA CAG CGT CGC A 3`). The amplification profile consisted of 2 minutes at 50°C and 5 minutes at 95°C followed by 45 cycles of amplification, each cycle consisting of denaturation at 95°C for 15 seconds, annealing for 20 seconds at 55°C and extension for 30 seconds at 72°C. Standard curves were generated in all experiments. PCR efficiency was assessed from the slopes of the standard curves and was found to be between 90% and 100%. Linearity of the assay could be demonstrated by serial dilution of all standards and cDNA. All samples were normalized for an equal expression of GAPDH.

***Statistical Analysis***

Numerical data are expressed as mean  $\pm$  standard deviation. For immunohistological parameters, renal function, PCR-analysis and histomorphometric analysis statistical analysis was performed using the Kruskal-Wallis test with option for multiple comparisons (StatsDirect 2.2.8, Aswell, UK). For analysis of the blood pressure data two-way ANOVA was applied. For analysis of light microscopy, Fisher's exact test was used. For survival analysis, Kaplan-Meier, Logrank and Wilcoxon tests were applied. A *P*- value of less than 0.05 was considered as significant.



## RESULTS

### *Influence of dopamine on mean arterial pressure and renal function in the donor*

Brain death induced profound hemodynamic alterations, which were characterized by an initial increase in mean arterial blood pressure (MAP), followed by sharp decline leading to persistent hypotension. Although in non BD animals MAP gradually declined during 6 hrs of ventilation, there was a significant difference in MAP observed between BD and NBD in the first 3 hrs (Fig 1 A).

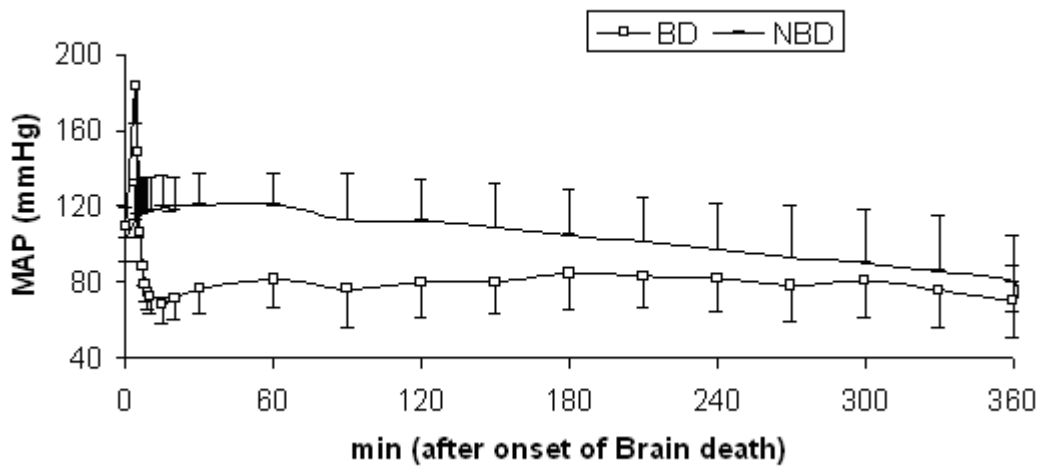


Fig 1 A: BD vehicle (open squares) in comparison to NBD group (black line). Although in non BD animals MAP gradually declined during 6 hrs of ventilation, there was a significant difference in MAP observed between BD and NBD in the first 3 hrs. The results are expressed as MAP (mmHg) of at least 4 animals in each group.

MAP in BD animals was completely normalized by installation of dopamine during this period or by infusion with HES (BD-normot). Cessation of dopamine infusion slightly decreased MAP compared to animals that were continuously treated with dopamine over the whole brain death period (Fig 1 B, C).

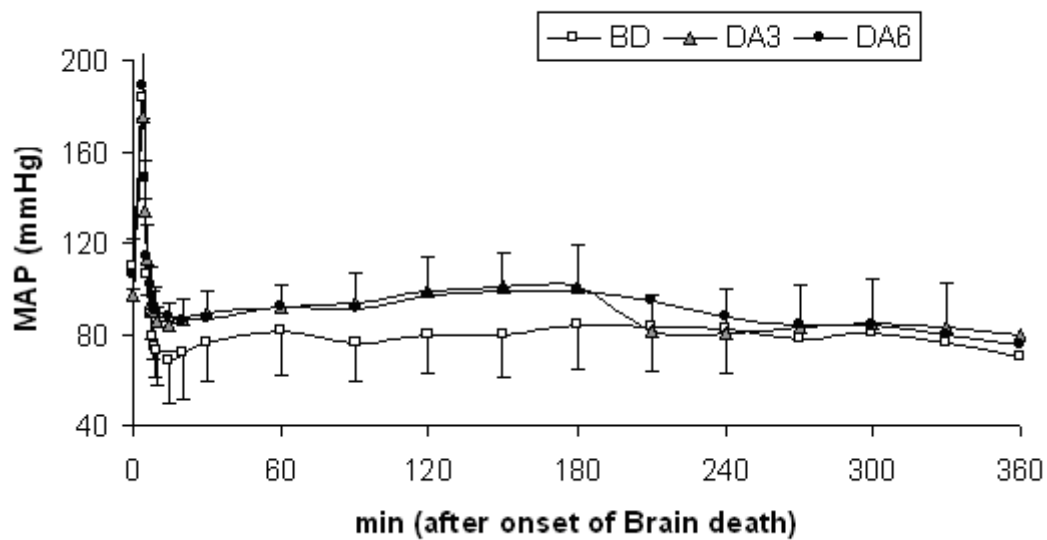


Fig. 1 B: Hemodynamic changes in BD donor rats. MAP was recorded as described in the method section. Dopamine treatment during BD (closed circles) significantly improved MAP compared to NaCl treated BD rats (DA6 vs BD,  $P < 0.05$ ). After cessation of dopamine treatment (DA3, grey triangles) MAP was not different from the NaCl treated BD rats (open squares). The results are expressed as MAP (mmHg) of at least 4 animals in each group.

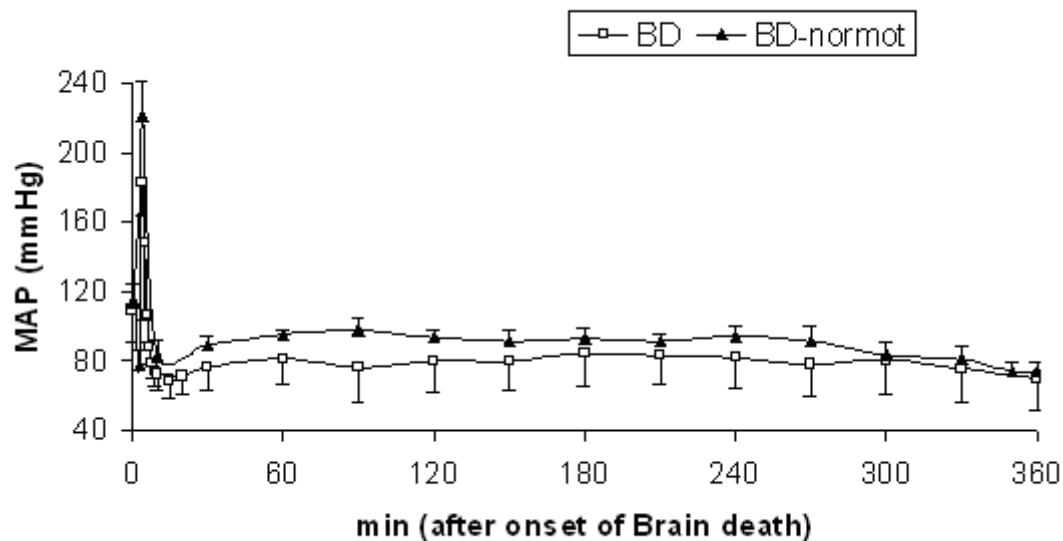
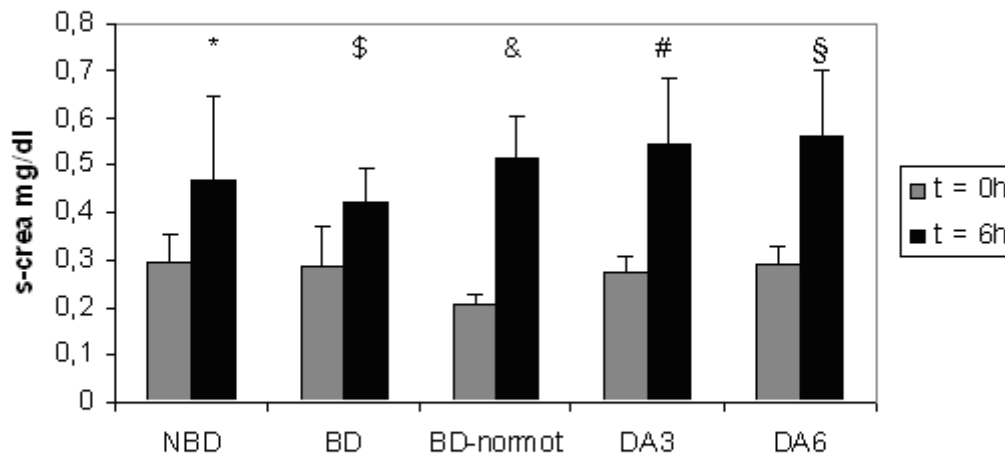


Fig. 1 C: BD vehicle (open squares) in comparison to BD normotensive group (closed triangle). HES-infusion stabilised blood pressure during BD. The results are expressed as MAP (mmHg) of at least 4 animals in each group.

Serum creatinine increased during BD in all donors. However, this was not in a pathological range. The rise in serum creatinine was not specific for BD as it also occurred in ventilated not BD donors. Serum creatinine was not significantly influenced by dopamine in the BD donors. (Fig. 1 D).



*Fig. 1 D: Serum creatinine (mg/dl) before and 6 hours after brain death induction in brain death donor rats or before and 6 hours after intubation in the living controls (NBD). Serum creatinine levels increased during 6 hours in all groups significantly. (NBD,  $t=0$  vs  $t=6$ :  $*P<0.05$ ; BD,  $t=0$  vs  $t=6$ :  $\$P<0.01$ ; BD-normot,  $t=0$  vs  $t=6$ :  $\&P<0.01$ ; DA3,  $t=0$  vs  $t=6$ :  $\#P<0.01$ ; DA6,  $t=0$  vs  $T=6$ :  $\$P<0.01$ ).*

*Each group consisted of 5 to 7 animals. Data are shown as means  $\pm$  standard deviation.*

***Donor dopamine treatment is associated with a better renal function in recipients***

The rise in serum creatinine one day after transplantation was significantly less in dopamine treated and NBD groups compared to vehicle treated BD rats ( $1.7 \pm 0.9$  vs  $0.8 \pm 0.4$  (DA3) and  $0.8 \pm 0.2$  (DA6), vehicle treated vs dopamine treated BD animals  $P < 0.05$ ). Although there was also a trend for better renal function at day 3 and 5 after transplantation this did not reached statistical significance due to the large standard deviation in the BD group. Serum creatinine in recipients which received a graft from dopamine treated brain dead donors did not significantly differ from that of recipients receiving a renal allograft from NBD animals ( $0.8 \pm 0.5$ ). Recipients receiving a graft from BD donors that were treated with HES showed a tendency for a decreased serum creatinine, but this was not statistical significant ( $1.7 \pm 0.9$  vs  $1.0 \pm 0.6$ , BD vs BD-normot,  $P = \text{NS}$ ) (Fig. 2).

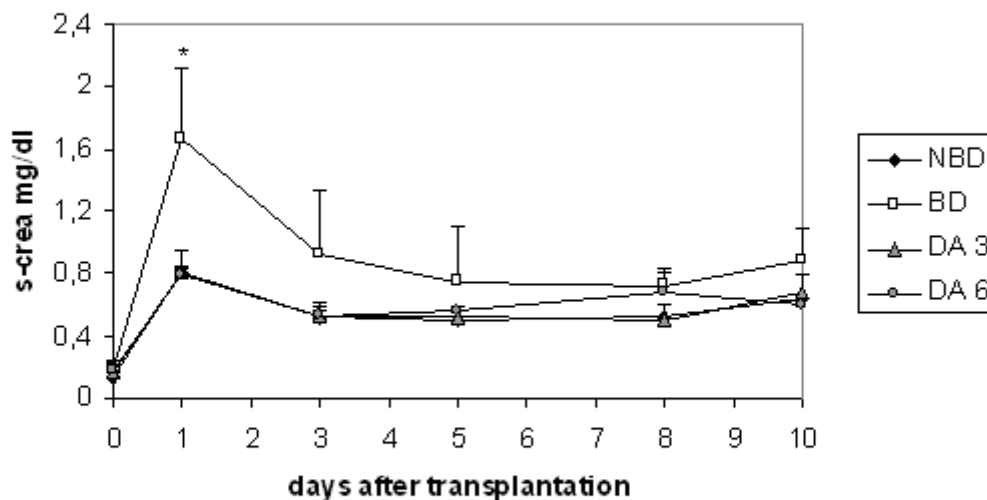


Fig. 2 A: Serum creatinine (mg/dl) on day 0, 1, 3, 5, 8 and 10 after transplantation. Dopamine treatment in brain dead donors significantly improved renal function in the recipients when compared to BD (BD vs DA3 and DA6,  $*P < 0.05$ ). Each group consisted of 5 to 7 animals. Data are shown as means  $\pm$  standard deviation.

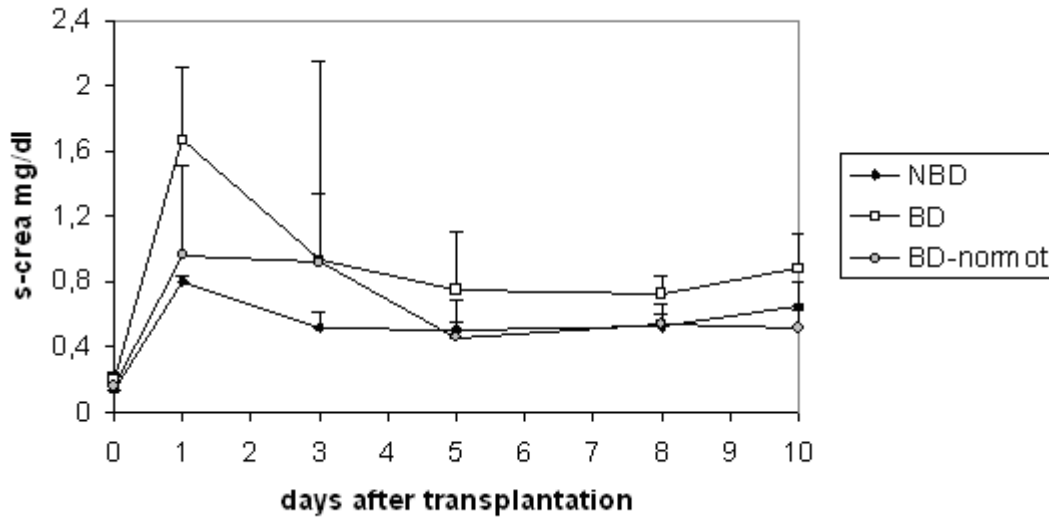


Fig. 2 B: The normotensive BD group showed a tendency for reduced serum creatinine, but this did not reach statistical significance. Data are shown as means  $\pm$  standard deviation.

### Banff-Classification

Light microscopic analysis according to the Banff 97 classification revealed a higher tubulitis and interstitial inflammation score in renal allografts obtained from BD animals (BD/BD-normot. vs NBD:  $P < 0.05$ , table 1). Tubulitis and interstitial inflammation was reduced in the DA3 and DA6 treated groups, although in the latter group this did not reach statistical significance (vehicle treated vs. dopamine treated (DA3) BD animals:  $P < 0.05$ , Table 1).

Banff	NBD (n=7)	BD (n=7)	BD- normot (n=5)	DA 3 (n=6)	DA 6 (n=6)
i0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
i1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
i2	4 (57.2)	0 (0)	0 (0)	4 (66.6)	2 (33.3)
i3	3 (42.8)	7 (100)	5 (100)	2 (33.3)	4 (66.6)
t0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
t1	4 (57.2)	0 (0)	0 (0)	4 (66.6)	2 (33.3)
t2	3 (42.8)	7 (100)	5 (100)	2 (33.3)	4 (66.6)
t3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
v0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.6)
v1	6 (85.7)	7 (100)	3 (60)	6 (100)	5 (83.3)
v2	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)
v3	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)

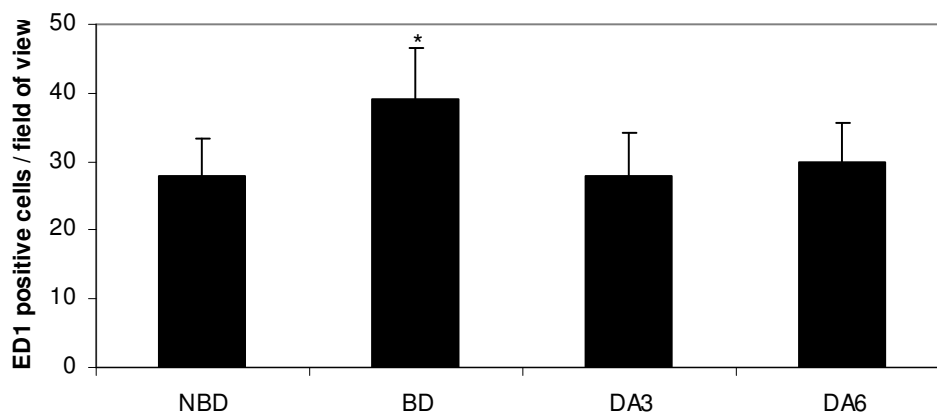
Table 1: Histologic scores according to the Banff 97 classification of allografts 10 days after transplantation. Living (NBD) or brain death (BD) donors treated with saline or with HAES respectively (BD-norm). In addition two groups of brain dead donors were treated with dopamine over 3 (DA3) and 6

hours (DA6) after onset of brain death. The results are expressed as number of animals with a

particular Banff'97 score; in parentheses, the proportion of animals with this score in each group is calculated and expressed as %. NBD and DA3 had significant less mononuclear cell interstitial inflammation and tubulitis ( $P < 0.05$ ). i, mononuclear cell interstitial inflammation; t, tubulitis; v, intimal arteritis.

***Donor dopamine treatment is associated with a reduction of monocyte infiltration in the recipient's graft***

In accordance to the Banff classification, the number of ED1 positive monocytes in renal allografts obtained from BD animals was significantly higher than in that obtained from NBD animals ( $39 \pm 7$  vs.  $28 \pm 5$  ED1 positive cells, BD vs. NBD animals,  $P < 0.01$ ) Donor dopamine treatment significantly reduced the number of ED1 positive monocytes ( $39 \pm 7$  vs.  $28 \pm 6$  (DA3) and  $30 \pm 5$  (DA6), vehicle treated vs. dopamine treated BD animals,  $P < 0.05$ ) (Fig. 3).



*Fig. 3 A: Analysis of ED-1 positive cells in renal allografts. Living donors (NBD) and brain death donors (BD) were treated with saline. Brain death donors of group 4 and 5 were treated additionally with dopamine over 3 or 6 hours from the onset of brain death. Renal allografts were transplanted into Lewis recipients. 10 days after transplantation the transplanted grafts were collected and analyzed. The number of ED1 positive cells was reduced in the grafts from DA 3 ( $28 \pm 6$ ) and DA 6 ( $30 \pm 5$ ) donors compared to BD vehicles*

(39±7) (DA vs BD,  $P < 0.05$ )

The results are expressed as mean number of positive cells per field of view  $\pm$  standard deviation. At least 120 fields of view were analysed comprising 6 to 7 animals per group. Analysis was performed using a magnification of 400x.

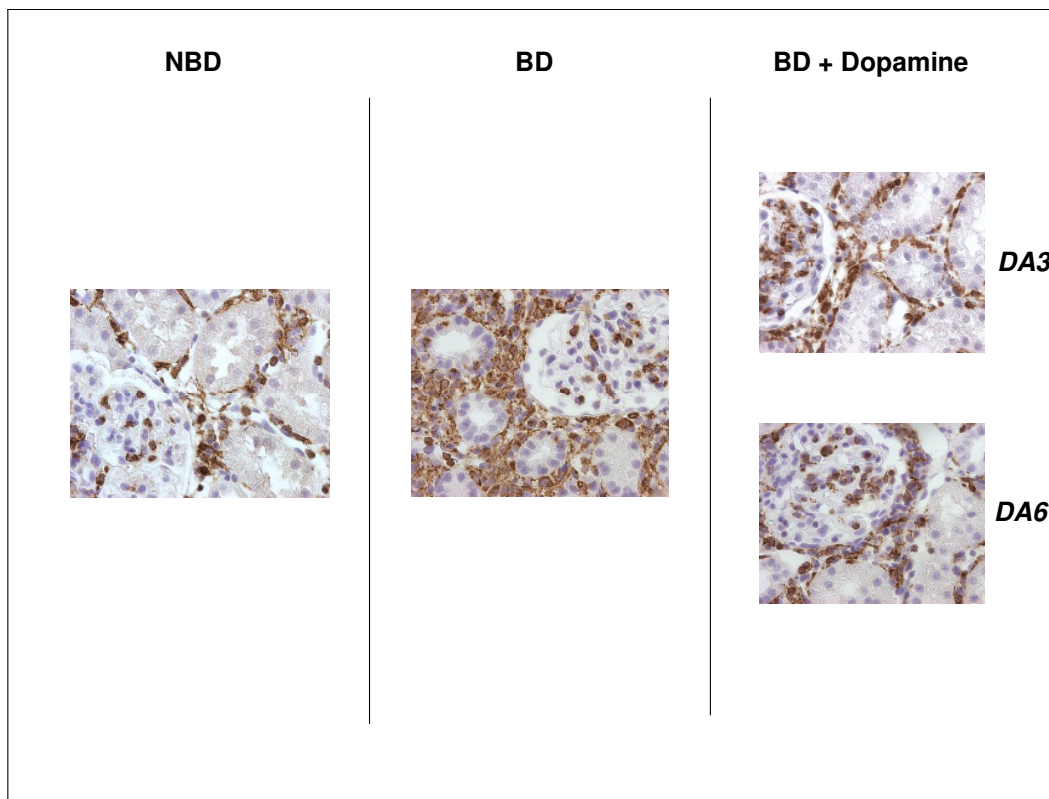


Fig. 3 B: Representative immunohistological staining for ED1+ cells in renal allografts collected 10 days after transplantation. ED1 expression obtained from NDB, BD, DA3 and DA 6 is depicted. Original magnification: 400x.

#### **Cytokine expression in the grafts 10 days after transplantation**

To investigate if the reduced renal inflammation in allografts obtained from dopamine treated BD animals was associated with a change in cytokine expression, IL-6, IL-10, MCP-1 and CINC-1 mRNA expression was assessed in these grafts. Although there was a tendency that IL-6 mRNA was decreased in renal allografts in the DA3 group, this did not reach statistical significance (IL-6/GAPDH ratio:  $9 \pm 5$  vs.  $4 \pm 2$ , vehicle vs. dopamine (DA3) treated BD animals,  $P=0.06$ ) (Fig. 4A). Likewise, MCP-1 mRNA expression between the groups was not

significant (MCP-1/GAPDH ratio:  $2406 \pm 210$  vs.  $2003 \pm 726$ , vehicle vs. dopamine (data not shown)). Also the increased IL10 mRNA expression in the dopamine treated group did not reach statistical significance (IL10/ GAPDH ratio:  $116 \pm 25$  vs.  $131 \pm 58$ , vehicle vs. dopamine (data not shown)). In contrast, CINC-1 mRNA expression was significantly decreased in the DA3 treated group (CINC-1/GAPDH ratio:  $80 \pm 25$  vs.  $34 \pm 15$ , vehicle vs. dopamine (DA3) treated BD animals,  $P < 0.05$ ). CINC-1 mRNA expression in this group was similar to that of the NBD group (CINC-1/GAPDH ratio:  $38 \pm 35$ ) (Fig 4B).

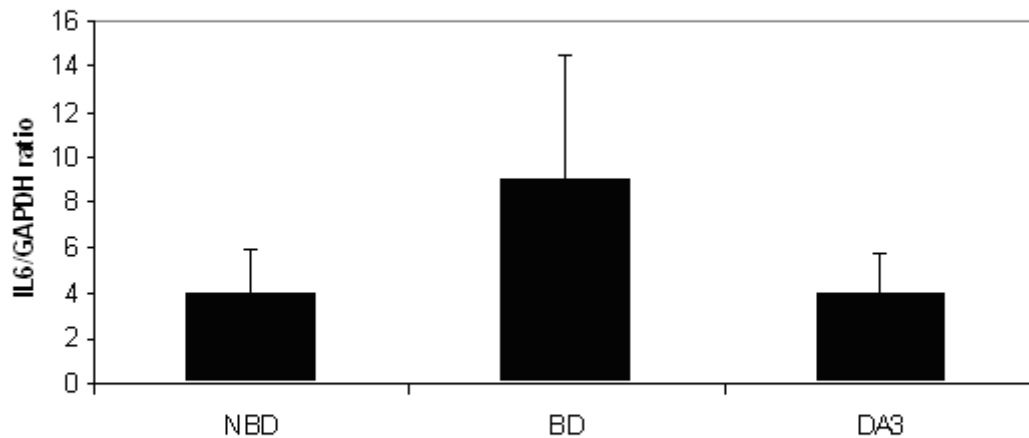
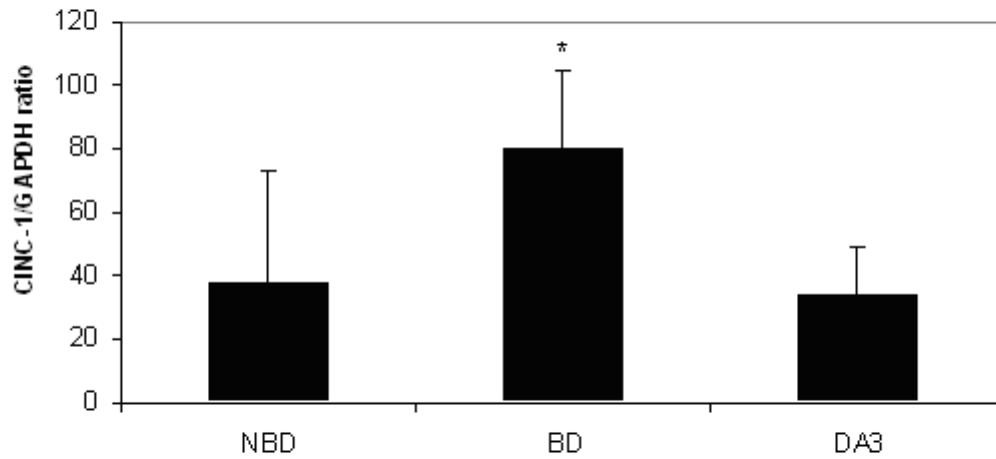


Fig. 4 A

*Fig. 4 Quantitative PCR-Analysis for IL6 (A) and CINC-1 (B) gene expression in grafts of ventilated non BD (NBD), NaCl treated brain death (BD) and dopamine treated brain death (DA3) animals 10 days after transplantation. The results are expressed as mean CINC1/S16 and IL6/S16 ratio  $\pm$  SD. In each group kidneys from 4 animals were analysed. CINC-1: BD vs DA,  $80 \pm 25$  vs  $34 \pm 15$ ,  $P < 0.05$ . IL6: BD vs DA,  $9 \pm 5$  vs  $4 \pm 2$ ,  $P = NS$ ; BD vs NBD,  $9 \pm 5$  vs  $4 \pm 2$ ,  $P = NS$ .*

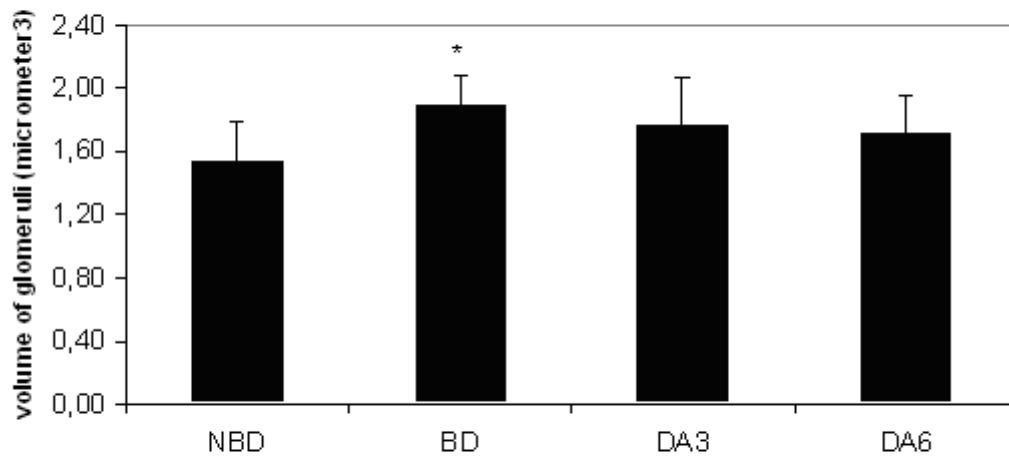




*Fig. 4 B*

#### ***Glomerular volume***

10 days after transplantation the glomerular volume was significant larger in grafts from BD compared to grafts from NBD donors (BD vs NBD:  $1.9 \pm 0.2$  vs.  $1.54 \pm 0.2 \mu\text{m}^3$ ,  $P < 0.01$ ). Donor dopamine treatment did not significantly influence glomerular volume (vehicle treated vs. dopamine treated BD animals:  $1.9 \pm 0.2$  vs.  $1.76 \pm 0.3$  (DA3) and  $1.73 \pm 0.3$  (DA6), Fig. 5).



*Fig. 5: Enlargement of glomerular volume. Ten days after transplantation glomerular volume in grafts from NBD animals was significantly less compared to grafts from BD rats (BD vs NBD:  $P < 0.05$ ).*

## DISCUSSION

Brain death is considered to be an important donor associated risk factor which influences organ quality [13, 15, 30]. Deterioration of organ quality might be related to a number of processes that can occur during brain death, e.g. hypotension, reduced organ perfusion, hypothermia, coagulopathies and inflammation in end-organs [1] [31, 32]. Since dopamine pre-treatment in donors reduces inflammation in donor renal allografts [22, 33], we investigated in the present study graft outcome in recipients that received renal allografts from dopamine treated brain dead donors. The main findings of this study are the following. Firstly, donor dopamine treatment improved mean arterial blood pressure, but it did not significantly influence renal function in the donor before harvesting. Secondly, renal function in the recipient was significantly better in rats receiving a renal allograft obtained from a dopamine treated brain dead donor if compared to the control rats. Thirdly, 10 days after transplantation the number of graft infiltrating cells was significantly reduced in the donor dopamine treated group. This was reflected by lower Banff 97 tubulitis and interstitial inflammation score.

In brain dead donors serum creatinine was slightly increased at the end of the brain dead period. This was however not related to brain death as it was also observed in ventilated non brain dead rats. Although dopamine increases renal blood flow, glomerular filtration rate, urinary sodium and water excretion [34], serum creatinine did not change in the brain dead donors in our study. In comparison to grafts obtained from untreated BD donors, renal function was significantly better 1 day after transplantation when the grafts were obtained from dopamine treated BD or NBD donors. Because renal function recovered in time, statistical significance disappeared, but there was still a trend for a better renal function in these two groups. We have chosen to include a group in which dopamine infusion was stopped after 3 hours to investigate if 3 hours of donor dopamine treatment was sufficient to influence renal function and inflammation in the recipient. We could already show that 3 hours of dopamine treatment was able to reduce infiltration of inflammation cells in BD donor kidneys significantly [23]. Indeed, 3 hours of dopamine treatment was sufficient to influence renal function and inflammation in the recipient beneficial. In the human situation, early renal function has an enduring effect on the subsequent course after renal transplantation [35-37] and predicts 5 years graft survival [38]. Improvement in early renal function by donor dopamine treatment might therefore significantly improve long term graft prognosis [19, 20]. It was surprising that some of the beneficial effects were observed for both dopamine treatment regimes, e.g. renal function in the recipients, while others, e.g. inflammation, were

only seen for 3 hours or 6 hours of dopamine infusion. Nevertheless if both dopamine groups were pooled, statistical analysis also revealed that in the dopamine treated groups there was significant less inflammation compared to the untreated BD group.

Hypotension might lead to reduced organ perfusion, tissue ischemia, and generation of reactive oxygen species (ROS) [13, 39]. Thus, hemodynamic stabilization before organ procurement can improve organ quality by limiting ROS mediated organ damage. Moreover, hemodynamic stabilization seems to reduce organ inflammation during brain death as was previously demonstrated [33] [11-13, 40]. Although it can be argued that improvement in blood pressure and organ perfusion largely contribute to the beneficial effect of donor dopamine treatment, in the present study we also demonstrate that other strategies to improve blood pressure during brain death were less effective or did not influence graft infiltration as assessed by serum creatinine and Banff classification respectively. Banff 97 classification revealed lower tubulitis and interstitial inflammation scores in the donor dopamine treated groups compared to the BD normotensive group. These findings therefore indicate that donor dopamine treatment can affect transplantation outcome independently of its hemodynamic effect. This is in accordance to the clinical findings of Schnuelle et al [41], demonstrating that the favourable effect of donor dopamine treatment was independent of donor blood pressure.

If improved hemodynamics only partially can explain the beneficial effect of donor dopamine treatment, then what other factors might be considered? Firstly, dopamine might ameliorate ischemia/reperfusion (I/R) injury as we have previously demonstrated [42]. Secondly, dopamine treatment reduces monocyte infiltration during brain death and hence reduces the number of passenger leukocyte in the graft [22, 33]. These mobile cells migrate out of the graft into secondary lymphoid organs where they can initiate an immune response against the graft [43]. While amelioration of I/R injury reduces renal inflammation and improves renal function, reduction in the number of passenger leukocytes might decrease the response to the allogeneic kidney [44]. Because tubulitis is a hallmark for acute interstitial rejection after renal transplantation in men, our data indicate that dopamine given to the donor may influence the process leading to acute interstitial rejection, as evidenced by a lower Banff tubulitis score.

Nevertheless, it remains to be further elucidated how exactly dopamine influences renal inflammation per se. Previously, we have shown that dopamine has the propensity to inhibit

IL-8 production in renal tubular epithelial cells [21, 45]. In the present study, we now demonstrate that CINC-1 expression, a rat homologue for IL-8, is significantly reduced 10 days after transplantation in the donor dopamine treated group. We also observed a tendency for reduced IL6 and MCP-1 expression in these grafts. A reduction in chemokine expression might also contribute to a decreased inflammatory response in the transplanted renal allograft.

Glomerular enlargement in renal allografts is associated with inferior graft survival [28] and with renal allograft dysfunction [46]. Ten days after transplantation glomerular size was significantly larger in grafts obtained from brain dead rats. Donor dopamine treatment however did not significantly influence glomerular size in these grafts.

The present study demonstrates that donor dopamine treatment during brain death may provide a benefit on graft survival both by improving early renal function after transplantation and by reducing renal inflammation. Our data are in concordance to the clinical studies of Schnuelle et al [20] who found that donor dopamine usage was associated with improved renal function, less acute rejection episodes and improved long term graft survival. These data are therefore justifying a prospective randomized multi-centre study on the beneficial effects of donor dopamine usage in terms of transplantation outcome.

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